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Correlations between Behavioral Changes and Drug Induced Variations of the Levels of Mouse Brain Catecholamines and Serotonin

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CORRELATIONS BETWEEN BEHAVIORAL CHANGES AND DRUG INDUCED VARIATIONS OF THE LEVELS OF MOUSE BRAIN CATECHOLAMINES AND SEROTONIN

by

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A dissertation submitted to the faculty of the Department of Pharmacology, Loyola University, Stritch School of Medicine, in partial fulfillment of the requirements for the degree of Master of Science

October 10, 1971

TO DR. CHARLES L. SCUDDER MY ADVISOR AND FRIEND,

THANK YOU..

TABLE OF CONTENTS

BIOGRAPHY

Daniel L. Richardson was born in Rockford, Illinois on October 30, 1942. He graduated from St. Thomas High School (Rockford) in 1961 and subsequently from Loyola University (Chicago) in 1965. During the last four years he has been a graduate student in the Department of Pharmacology at Stritch School of Medicine.

Chapter I

INTRODUCTION

Evidence for Chemical Transmission

Over the past twenty five years there has been an accumulation of much data which provides evidence for chemical transmission in the peripheral nervous system (Dale, 1938; Katz and Miledi, 1962; Hebb and Smallmon, 1957; Whittaker and Sheridan, 1965; Wilson and Ginsburg, 1955; Krupa, 1963; Von Euler, 1966; Curtis, 1964). However, data pertaining to the role of chemicals as transmitters in the central nervous system (CNS) has been more difficult to accrue due to the anatomical and physiological complexities of these parts (Eccles, 1964).

Generally, satisfaction of the following criteria is considered proof of transmitter function:

1) The proposed substance must be identified as being present in the neural tissue in question; 2) The substance must be released and must produce a physiological effect upon stimulation; 3) An enzymatic system of synthesis and degradation must be present to produce a constant supply of the substance; 4) There should be storage mechanisms present in the area in question; 5) Neurons receptive to the substance must be demonstrated - and finally; 6) Pharmacological analysis should demonstrate a similarity between exogenously applied and endogenously released transmitter substance.

When the data relevant to the transmitter role of a compound such as norepinephrine in the central nervous system is compared with that of

acetylcholine at the peripheral synapses, the proof of the transmitter role of norepinephrine centrally is not conclusive.

This evidence suggesting a central transmitter role for norepinephrine includes the following observations: 1) There are regional brain differences in the distribution of norepinephrine which appear to be related to certain neurophysiological sub-systems of the nervous system (Rech et al., 1966; Van Rossum, 1967). 2) High levels of the enzymes related to the synthesis of norephinephrine are associated with structures that look like synaptice vesciles (Michaelson et al., 1963; De Robertis, 1966). 3) By fluorescent techniques catecholamines have been localized to the CNS (Dahlstrom and Fuxe, 1964; Fuxe, 1965; Falck et al., 1962). 4) Fluorometric analysis of whole brain amines (Shore and Olin, 1960; Weigen and Pery, 1961; Everett, 1961) have also documented the presence of catecholamines centrally. 5) Induced neural activity changes brain norepinephrine levels (Reis and Gunne, 1965). 6) Microiontophoresis and intraventricular administration of the amines and substances relating to their synthesis and degradation have electrophysiological and behavioral effects.

There are many technical problems involved with any attempt to use the peripheral nervous system as a model for studying a possible transmitter in the central nervous system. The dendrites are numerous and small, and packed within a tangle of glia. This makes isolation and chemical and physical manipulation very difficult. There are, of course, neural tracts and specific areas of known physiological activity (Fuxe et al., 1965). However, the separation and identification of separate

neuron cell bodies is extremely difficult.

It is difficult but possible to obtain by activation of neural tracts and areas in the central nervous system discrete, replicable, physiological phenomena such as the miniature end-plate potential obtained by suitable electrode placement in the peripheral nervous system (Eccles, 1964).

Catecholamines as Modulators

The administration of drugs which have been found to affect levels of the amines in the central nervous system and which also affect behavior has led to numerous speculations based on a supposed transmitter and/or modulator role of the catecholamines and serotonin (Everett, 1961; Everett and Weigard, 1962). These speculations often are present as hypothesis relating behavioral states to the adrenergic, cholinergic, dopaminergic, serotonergic, etc., "systems of the brain". An investigation into the role of adrenergic and serotonergic systems in behavior is the basis of the research presented in this thesis.

Catecholamine Metabolism

Catecholamines are low-molecular-weight substances which contain a catechol nucleus and an amine group. The more commonly studied catecholes are dihydroxyphenylalanine (dopa) and its metabolic products, dopamine, norepinephrine and epinephrine. These compounds are synthesized from their precursor, tyrosine by a known series of enzymatic reactions. The amino acid tyrosine, normally present in the circulation, is concentrated in neural tissue by an active transport mechanism. Intraneuronally tyrosine undergoes three specific enzymatic transformations which result

in the production of norepinephrine. In those cells which contain the enzyme needed to snythesize epinephrine, there is an additional intracellular migration of norepinephrine, from the granule to the cytoplasm for N-methylation, and then back again to the chromaffin granule for storage. The following is a diagram which illustrates the biosynthesis of catecholamines in brain, chromaffin cells (adrenal medulla) and sympathetic nerve endings (Fig. 1).

Serotonin Metabolism

Serotonin (5HT) is an indol amine which is synthesized in the brain from the amino acid tryptophan. The following is a diagram which illustrates the biosynthesis of serotonin (5HT) (Fig. 2).

The catecholamines are degraded by the enzymes monoamine oxidase and catechol-o-methyl transferase. Serotonin is also degraded by the enzyme monoamine oxidase. Drugs which affect each of these enzymes will be considered in the following chapter as they affect the brain levels of the amines and serotonin (Chapter II, d).

Catecholamines, Serotonin and Behavior

The causal effects of brain catecholamine levels and serotonin levels on behavior have not been clearly substantiated. The following statements are examples from published papers. "A case can be made for the catecholamines having either central excitant or central depressant actions" (Marley, 1966). "With the facts so far available it is not possible to say what function dopamine has in the central nervous system", (Bertles and Rosengren, 1966). An still in reference to psychiatric states in humans, the evidence as a whole does not permit assigning importance to

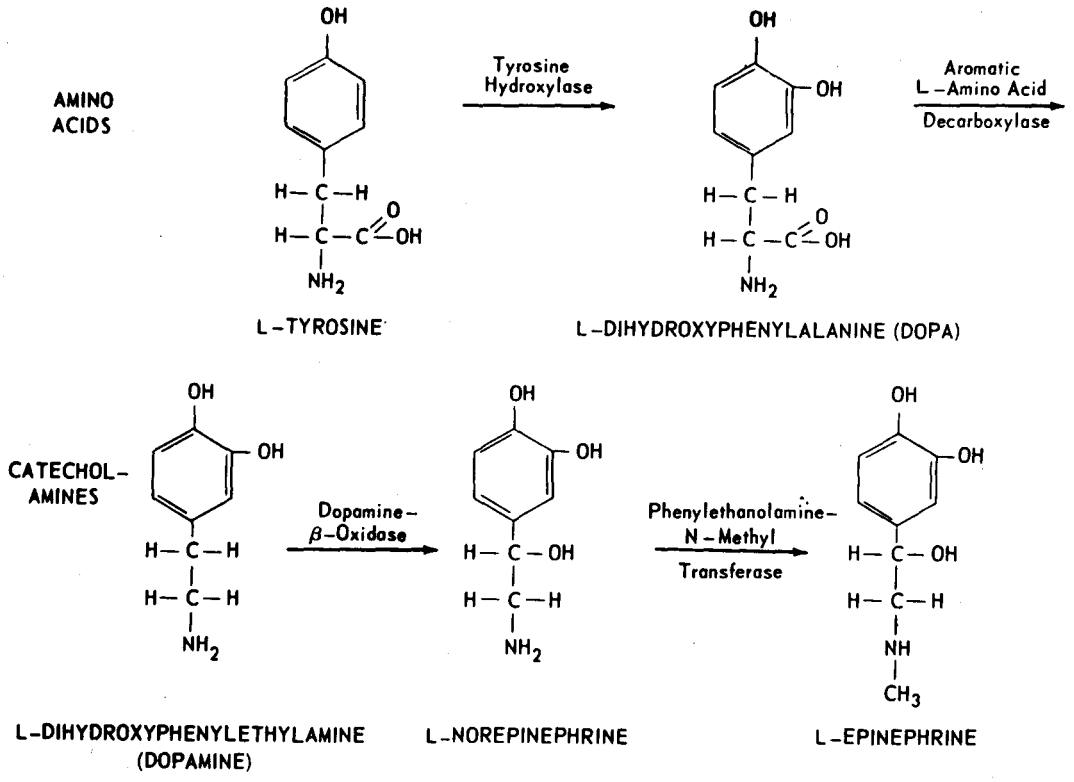
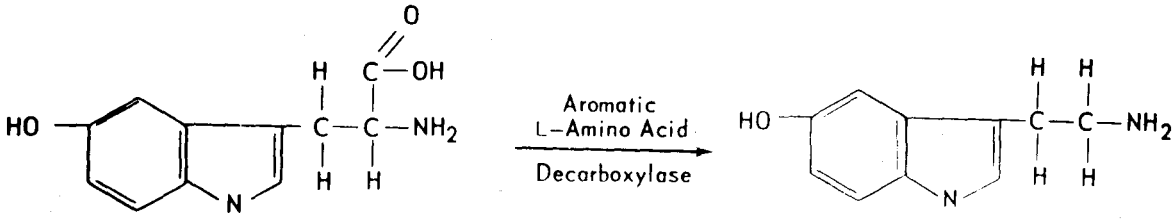


Fig. 1

Biosynthesis of Catecholamine



5-HYDROXYTRYPTOPHAN

5-HYDROXYTRYPTAMINE
(SEROTONIN)

Fig. 2

Biosynthesis of Serotonin

the catecholamines specifically" (Kety, 1966).

Dr. Dews in a symposium on catecholamines stated that studies of the effects of Biogenic Amines on Operant Behavior show that the complex behavioral output of an intact animal can be effectively used to study drug interactions and antagonisms. He concluded, however, that "studies of transmitters and related compounds have shown that patterns of behavior as a whole tend to be suppressed or enhanced, without specific reference to individual components of the behavior" (Dews, 1968).

This attitude is not shared by all investigators, however. Frequently a direct relationship is implied when a behavioral state is found along with altered amine levels as the result of drug administration. A direct correlation for example between psychosis and serotonin is presumed by Corrodi (1966). "Thus the high excess of 5HT in the mouse brain seen after nialamide treatment seems to be predominantly responsible for this model psychosis".

Some drugs which significantly alter the brain levels of biogenic amines in animals also produce changes in behavior. A classical example of this is reserpine which produces many behavioral effects, such as sedation, a loss of conditioned avoidance, a decrease in self stimulation, and a behavioral state in rats analogous to clinical endogenous depression in man (Kety, 1967a). A remarkable correlation persists between the action or reserpine and its effect on catecholamines suggesting that the action of reserpine on behavior is dependent on its effects on the central amines (Kety, 1962).

Because drugs such as reserpine and monoamine oxidase inhibitors affect

more than one amine similarly, they have not been useful in relating particular amines to their behavioral effects. Other drugs such as specific precursors, and specific inhibitors have been of greater value. There will be discussed in detail with regard to their pharmacological activity and effects on behavior in the following chapters.

α-Methyl-p-tyrosine, which blocks tyrosine hydroxylase and lowers brain catecholamine levels but not at all 5HT levels, has been reported to produce sedation in man (Sjoerdsma et al., 1965).

P-Chlorophenylalanine, which blocks the synthesis of 5HT in the brain without affecting other amines such as norepinephrine or dopamine, has been examined for its effects on behavior. It does not appear to produce sedation even though 5HT is reduced to minimal levels in the brain (Koe and Weissman, 1966).

Most drugs which rather specifically alter affective states in man and behavior in animals have been shown to interact in some manner with biogenic amines in the brain.

Reserpine which was used in psychiatry in the treatment of mania and excitement prior to the introduction of the phenothiazines and is used in general medicine in the treatment of hypertension has been reported to produce severe depression of mood in some patients, particularly hypertensive patients treated with relatively large doses (Glowinski et al., 1965). Depression has also been observed in patients given tetrabenazine, a drug which is similar to reserpine in its effects on biogenic amine metabolism (Lingjaerde, 1963).

The effects of reserpine have been extensively studied in experi-

mental animals and in-vitro systems. By a mechanism which has not yet been completely elucidated, reserpine interfers with the intraneuronal binding of the catecholamines and serotonin (Carlsson et al., 1957). With the binding thus impaired the amines may diffuse freely through the cytoplasm and attach to mitrochondrial monoamine oxidase. This results in their inactivation by deamination and thus, in depletion of tissue amine stores. Reserpine-induced sedation in animals is associated with decreased brain levels of norepinephrine, dopamine and serotonin (Carlsson et al., 1963).

There have been reports of a temporal association between the return of normal motor behavior in animals sedated with reserpine and the restoration of the brain's capacity to accumulate both norepinephrine injected into the cerebral ventricles and serotonin synthesized from the exogenously administered precursor 5-hydroxytryptophan (Brodie et al., 1966). These findings support the hypothesis that reserpine induced sedation is related to impairment of the binding of monoamines, but the data do not allow separation of the effects of catecholamine depletion from those of serotonin release or depletion. However, relevant data come from studies in which the amino acid precursors of catecholamines and serotonin have been administered to animals previously given reserpine. These precursor amino acids, unlike the monoamines, can cross the blood-brain barrier in animals and raise the concentrations of the respective monoamines in the brain (Carlsson et al., 1957). Administration of dihydroxyphenylalanine, the catecholamine precursor, reverses reserpine-induced sedation in animals and restores gross behavior to approximately normal levels (Carlsson et al.,

1963; Everett and Toman, 1959). The serotonin precursor, 5-hydroxytryptophane, however, does not restore normal functioning (McGeer et al., 1963). These findings suggest the importance of catecholamine depletion in reserpine-induced sedation in animals, and possibly depression in man.

Based on the studies of Everett, Carlsson and others dopamine is now emerging as an important neurotransmitter in its own right. Previously dopamine was thought of primarily as a precursor to norepinephrine with little action of its own. Dopamine in some cases is better correlated with changes in behavior than norepinephrine (Creveling et al., 1968). Along this same line interest in dopamine as a neurotransmitter has been fostered by Hornykiewicz (1966) who showed a deficit of dopamine in basal ganglia of parkinson patients.

L-Dopa, the precursor to dopamine, is now widely used in the treatment of parkinsons disease (Boullin and O'Brian, 1970). When theorizing about the possible function of neurotransmitters one must not talk of catecholamines in general but must distinguish between the two amines, norepinephrine and dopamine.

A neurochemical theory, the catecholamine theory of mood, states that clinical depression is associated with a functional deficiency of central norepinephrine and, perhaps, the inverse in states of mania. Practically all the drugs that influence mood in man have been associated with this theory in spite of the fact that, without exception, none of them acts on norepinephrine levels only. They include cocaine, amphetamines, monoamine oxidase inhibitors, lithium, phenothiazines, and metabolic inhibitors.

However, as far back as 1914, research has verified that epinephrine or norepinephrine, when given so that it passes the blood-brain barrier (that is, intraventricularly or intravenously into animals with immature blood-brain barriers), produces a dose-response pattern of behavioral depression, lethargy, sleep, sedation, and stupor (Boss, 1914; Morinesca et al., 1929; Leimdorfer and Metzner, 1949; Sherwood and Richter, 1955; Rothballer, 1959; Milhand and Glowinski, 1963; Brittain, 1966). These and other contradictory reports in both man and animals have lead to these conclusions: "At the present time, the neuropharmacological psychochemical research approach rests on insecure bases both in terms of the establishment of what are necessary and sufficient conditions in brain chemistry to explain the metabolic effects of the drugs and in terms of the connection of these changes with predictable behavioral phenomena" (Mandell and Spooner, 1968).

The effects of amine levels generally are apparent only when the levels are greatly changed. Norepinephrine must be almost depleted before the behavioral effects of depletion are seen. "The actual content of amines of the tissues is of only minor importance for the function" (Carlsson et al., 1963).

Behavioral states induced by drugs such as L-dopa, phenethylamine, 5-hydroxy-tryptophane, and tryptamine have been all lumped as one "psychosis" although obviously different to a careful observer (Randrup and Munkvad, 1966). Surely, if a direct correlation exists between levels of brain amines and behavior, the behavioral symptoms resulting from general accumulation of depletion of amines by different drugs should be similar.

This has generally not been the case, however, based on the work done in this area.

In studies in which drugs are not employed, correlations have been found between amine and serotonin levels and emotion (Pryor et al., 1964), stereotypy and shock latencies (Scudder et al., 1968), and audiogenic seizures (Schlesinger et al., 1965).

Some affective states in animals are accompanied by great alterations in the central catecholamines. Welch and Welch (1965) found a lower brain norepinephrine content in mice which were isolated than animals kept in aggregated groups of 25. Maynert and Levi (1968) found a 40% reduction in brain norepinephrine concentration in rats following electrical shock to the feet. Barchas and Freedman (1963) found a fall in Brain NE levels and an equivalent rise in brain 5HT levels following a swimming stress in rats. Liberson (1964) reported a significant change in brain 5HT content as a result of behavioral stress such as fixation. If external sensory inputs such as those arising from stress, shock, isolation and training modifies amine levels, it might be expected that any drug which induces internal change sensed by afferent nerves which record an animal's internal environment might produce a change in amine and serotonin levels by the same mechanism. The amine levels may not be a cause of behavior but a result of a sensory stimulus.

The strength of drug effects on animal behavior varies depending on the excitability of the central nervous system regardless of whether or not amines are affected by the drugs (Souskovi, 1964). Furthermore, drugs affect specific behaviors in ways quite apart from simple changes in rates

of task performances or amine level changes; in other words, drug effects are related to specific temporal sequences or responses and stimuli which constitute behavior and over which amine levels may change in a non-specific way (Kelleher et al., 1964).

As a result of recent advances in Radioactive tracer methodology the rate of synthesis or so called "turnover rate" of catecholamines and serotonin has been determined (Costa and Neff, 1969; Neff et al., 1969). There are various methods used to determine the "turnover rate" of catecholamines and serotonin. These include In Vivo Studies and In Vitro Studies. The use of radioactive labeled precursors, ^{14}C tyrosine and ^{14}C tryptophane, in in vitro and in vivo studies is based on the fact that ^{14}C has a long half life; 5720 years and that ^{14}C will remain attached to the tyrosine or tryptophane molecules as metabolism of the amino acids proceeds to the products norepinephrine and serotonin respectively (Wang and Willis, 1966). Thus a radioactive product is formed which can be identified by means of liquid scintillation (Costa and Neff, 1969). ^{12}C is the normal carbon atom attached to the monoamines. The ^{14}C can be attached biochemically at various carbon positions, e.g., C 1, 2, or can be uniformly attached to the amino acid. The attachment of the ^{14}C does not interfer in any way with the metabolism of tyrosine or tyrptophane nor does it interfer with its function. Although radioactive labelled precursors are expensive they are readily obtainable and relatively safe to use.

The determination of "turnover rates" of catecholamines and serotonin has added some information as to the function of these biogenic

amines in the central nervous system. Recent studies have shown significant changes in "turnover rates" of biogenic amines associated with drug treatment and other parameters. Hery et al. (1970) for instance showed an increased synthesis and utilization of serotonin in the central nervous system of the rat during paradoxical sleep deprivation, implicating serotonergic mechanisms may play a role in the triggering of paradoxical sleep. Essman (1968) found that isolated mice showed a lower turnover rate and higher turnover time of brain serotonin, as compared to group-housed animals; this finding suggested a decrease in brain serotonin synthesis and inactivation for mice isolated over a prolonged period of time. It was suggested that the changes in brain serotonin metabolism may in this instance account for the behavioral differences found between isolated and group-housed mice.

Tricyclic antidepressants are effective in the treatment of some depressions, and it has been suggested that the clinical effects of these drugs may be related to their effects on biogenic amines in the central nervous system. Schildkraut et al. (1971) found the turnover of norepinephrine was decreased after acute administration of tricyclic antidepressants but increased during chronic administration of these drugs. This increase in norepinephrine turnover occurred sooner when thyroxine was administered with the antidepressant. The suggestion was made that this may help to explain why clinical antidepressant effects are generally observed only after chronic administration of imipramine or protriptyline and why thyroid hormone may accelerate and enhance the clinical antidepressant effects

of imipramine.

It seems true that metabolic paths relating to the amines are very labile because almost any stress in the environment or internal millieu causes fluctuations in the amines. The purpose behind these experiments is to study the amines and behavior in an attempt to find a correlation between the two, and to discuss a possible mechanism of action responsible for the changes in behavior. The research was not intended to prove or support a tight causal transmitter actuated behavioral mechanism, but it can be seen from the above brief survey that there is a need for studies on the relationships between amine and serotonin levels and behavior. This research is extremely important both to clear the air of needless suppositions and hypothesis and to provide a comprehensive ground plan to guide further research. In defense of the resulting conclusions the author would like to quote the following: "Even on a neurochemical level, we must talk about emperical correlates rather than determinents of behavioral states. Rigorous establishment of the transmitter or modulator role of brain substances appears to remain for the future" (Mandel and Spooner, 1968).

Chapter II

MATERIALS and METHODS

Animals

All the animals used in these studies were male, 60 ± 10 day old, ICR mice purchased from Scientific Small Animal Laboratory. Prior to the experiments the animals were housed for at least three weeks, fifteen to a cage, in an environmental chamber at 75 degrees ± 5 degrees F with an 8 hour light cycle. The animals were fed on Purina mouse pellets and water available *ad libidum*.

Behavioral Test

The environmental situation was a "Mouse City" set-up made of plexiglass. It is composed of six small home cages (5" x 7" x 4") each connecting with a central communal chamber (18" x 11" x 6") by means of a funnel-like, 12" x 1½" cylindrical runway made of plastic tubing (Fig. 4). Illumination was provided for all chambers by a diffuse overhead 40 watt red light. Sawdust was placed on the floor of all the chambers, and food pellets and water were provided in the central chamber.

In the city experiment, two aggregated males, which were naive to one another, were weighed; the animals were marked with dye for the purposes of identification, and after suitable drug pre-treatment one pair was placed in each of the small "home" cages of the "city". The experiment proper was initiated fifteen minutes later, at which time cotton plugs separating the "home" cages from the communal chamber were removed so that the animals were free to interact. At five minute intervals the behavior

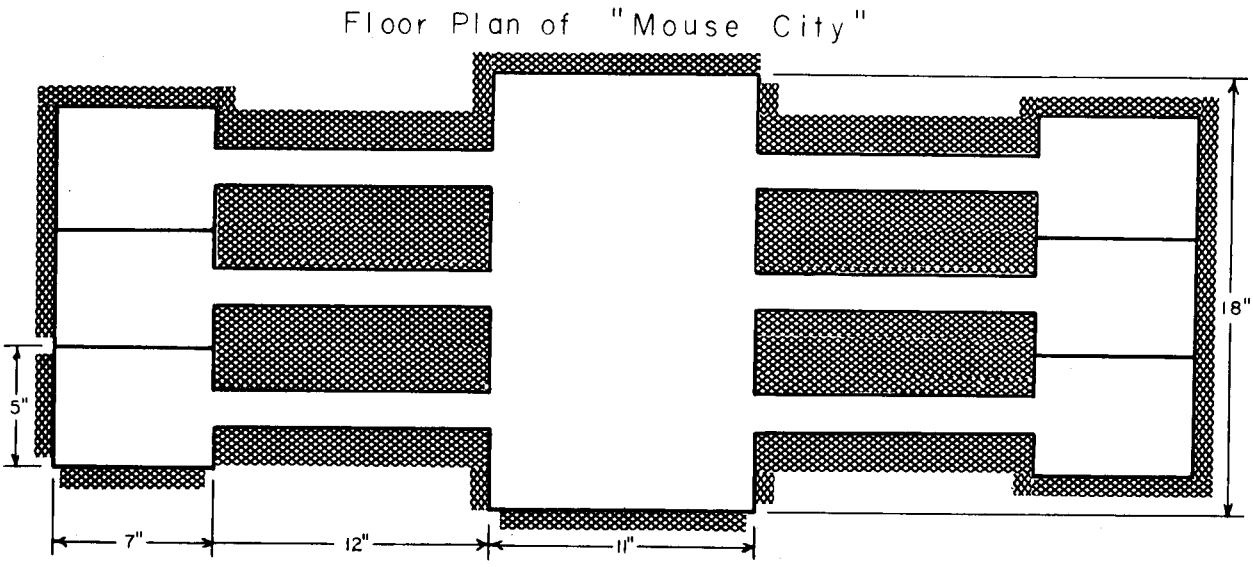


Fig. 4

Diagram of Mouse City

of each mouse at that time was recorded on a tape. Specific, carefully defined, behavior patterns (e.g. exploration, digging, stereotypic behavior, food-carrying, sexual behavior, etc.) were identified. Aggressive encounters were recorded at any time throughout the experiment.

Each "city" study was run for a total of seventy-five minutes (plus the initial fifteen minute pre-experiment period). Thus, fifteen five-minute checks per experiment per mouse were made, each corresponding to a distinct behavioral trait. Altogether, twenty "city" experiments, each using naive mice, were conducted per drug group. Twenty saline control cities were also conducted. The mouse "city" experiments were run in the afternoons between one and four o'clock.

The behaviors measured in the "city" were defined as follows: Motor movement from any one chamber to another, devoid of aggression and distinct from rapid flight, was recorded as exploration. Any other motor activity concerned, for instance, with fighting or gathering food, was not recorded as exploration. Digging consisted of clawing at, piling up or re-arranging the sawdust. Carrying things was defined as transportation of food pellets, feces, sawdust, etc., from one point to another, whether or not it was subsequently eaten. Attack (aggression) was defined as a movement toward another animal accompanied by a bite. An animal was listed as attacked if it was bitten. An attack was recorded as ended when the attacker released the attacked. (The separation before renewal of an attack was often brief.) Sexual behavior was defined as mounting, accompanied by pelvic thrusts. Sterotypic behavior consisted of any repeated act which seemed to be self-rewarding and purposeless and which did not seem to be

an effect of the environment (e.g. repeated small jumps in one spot, sommersaulting, running in circles, etc.). In the act of freezing mice became motionless and rigid; slight tremors were usually noticed and often they "froze" in a semi-erect position, i.e., on their hind legs. This behavioral trait could be readily differentiated from resting or sleeping; it was generally, but not always, a response to a disturbance. Sleeping animals were usually found in a curled up position, their eyes were closed and their breathing was slow, deep and regular. Ingestion was defined as any intake of food or water. Self grooming and grooming other mice consisted of the animal contacting gently with its mouth, tongue, teeth or paws either its own body or that of another animal, respectively. The term being groomed is self explanatory. Any unique or unusual trait (e.g. catatonia) was also recorded at the five-minute check period.

Biochemical Determination **

The measurement of the biogenic amines was by means of an Aminco Bowman Spectrophotoflurometer according to the technique of Wiegand and Perry (1961). Four pooled whole mouse brains were homogenized in two volumes of 0.01 NHCL and extracted using four ml of homogenate, two gm of NaCL and twenty-five ml n-heptane into four ml of 0.01 N HCL. In each extraction the tubes were shaken at 250 counts/minutes for ten minutes. Aliquots of 0.6 ml of this final acid extract were analyzed for norepinephrine and epinephrine using pH 5 and pH 3 buffer respectively. Fluorescence

** A special thanks to Dr. Guy Everett from Abbott Laboratory for the use of his lab, technician, and equipment while I was learning the fundamentals of the biochemical technique.

was measured in the spectrophotofluorometer at a wavelength of 397 mu and 508 mu.

Of the acid extract 1.0 ml was placed on a water-washed Dowex 50 x 4 (200 - 400) mesh, Na-form column, 2 mm high and 5 mm wide. The sample was washed with a single application of 2 ml of water. The dopa was eluted in one tube with 8 ml of 0.025 M sodium phosphate buffer pH 6.5, and the dopamine was eluted in a second tube with 8 ml of the same buffer containing 1 MKCl. Because fluorescent intensity developed slowly in these samples unless they were irradiated fluorescence was determined 22 hours later at activation and fluorescent wavelengths of 334 mu and 380 mu respectively.

The remainder of the acid extract was used for the analysis of serotonin by measuring fluorescence of the extract directly at the wavelengths of 300 mu and 342 mu. An acid blank and standard solutions of dopa, dopamine, norepinephrine, epinephrine and serotonin were subjected to the same manipulations as the samples.

Thus, during one determination the brain levels of dopa, dopamine, norepinephrine, epinephrine and serotonin were analyzed. The concentrations of the biogenic amines and serotonin are given as μg/gm wet weight in Table II, and as percent change from control in subsequent experiments.

Recovery experiments were conducted prior to the present set of experiments; 90% of the norepinephrine, 71% of the dopa and 75% of the dopamine standard added to control aliquots were recovered. Approximately 75% of the serotonin was recovered. Since these readings were constant over a series of experiments and since the concentrations in brain are

given as percent change from control brain values, recovery experiments were not conducted with each determination. However, a saline control was conducted with each set of experiments, and the values for the amines were almost identical in this case.

The mice used for the biochemical determinations were taken from the "mouse city" at the mid point of a regular "city" experiment (i.e., 35 minutes after the initiation of the experiment). Behavioral determinations were not recorded for these experiments. The levels of amines of the sacrificed mice were presumed parallel to those of the mice which were kept for a complete "city" run and whose behavior was quantitated. The animals used for the biochemical determinations were taken from the environmental (city) situation to eliminate any variable due to possible effects of this environment on biogenic amines and serotonin (cf. introduction). Although the behavior of the sacrificed mice piror to their deaths was not recorded, it was judged similar in all ways to the corresponding control or drugged "city" mice.

Drugs

All drugs were made up in saline and administered i.p., in a 0.01 ml/gm of mouse weight volume.

Two mg/cc of tragecanth were added to suspend (CP10) parachlorophenylalanine and (AMPT) alpha-methyl-paratyrosine in saline. Certain abbreviations are used in this paper as follows:

CNS = central nervous system

NE = norepinephrine

E = epinephrine

α MPT = methyl-para-tyrosine

Cp10 = parachlorophenylalanine

MAOI = mono-amine oxidase inhibitor

5HTP = 5-hydroxy tryptophane

5HT = (serotonin) 5-hydroxy trypt amine

L-DOPA = dihydroxyphenylalanine

DA = dopamine

Mechanism of Action of Drugs Used

(Fig. 3)

α-methyl-para-tyrosine (MPT)

αMPT inhibits the enzymatic formation of Dopa from tyrosine. Specifically it is a potent inhibitor of tyrosine hydroxylase and as such reduces brain catecholamine levels (Moore, 1967). Since αMPT does not affect brain 5HT levels, this agent is a valuable tool for investigating the significance of catecholamines in the central nervous system (Spector et al., 1965).

This drug has been extensively used as a pharmacological tool for the investigation of brain catecholamines (Moore, 1968; Rech et al., 1966; Levitt et al., 1965).

Pargyline (MO-911)

The enzyme monoamine oxidase is responsible for the degradation of the

DRUG	DOSAGE	Mech. of Act.
Pargyline (MO-911)	100 mg/kg	MAO Inhibitor
L-Dopa	50 mg/kg	NE Precursor
5-Hydroxytryptophane (5-HTP)	100 mg/kg	5HT Precursor
α-methyl-p-tyrosine (α-MPT)	80 mg/kg	Tyrosine Hydroxylase Inhibitor
P-chlorophenylalanine (CP-10)	316mg/kg	5HT Synthesis Inhibitor

Fig. 3

Drugs, Dosage and Mechanism of Action

catecholamines and serotonin. This enzyme, along with catechol O-methyl transferase (COMT) plays a key role in controlling the amounts of NE, 5HT and DA stored in the brain (Spector et al., 1960). Pargyline inhibits the enzyme monoamine oxidase so that its administration leads to a marked increase of endogenous amines and serotonin (Shore and Olen, 1958; Spector et al., 1960; Quastel, 1965; Wiegand and Perry, 1961).

(P-chlorophenylalanine) (CP-10)

P-chlorophenylalanine blocks the synthesis of 5HT in the brain without affecting other amines such as norepinephrine or dopamine (Koe and Weissman, 1966).

P-chlorophenylalanine inhibits liver tryptophane hydroxylase *in vitro* and strongly suppresses the tryptophan and phenylalanine hydroxylating capacities of liver treated with it (Koe and Weissman, 1966). These results suggest that P-chlorophenylalanine may effect 5HT depletion by inhibiting the biosynthesis of this monoamine probably by blocking tyrptophan hydroxylation. It has also been suggested that Cp10 may interfere with uptake of the precursor 5HTP (Pletscher et al., 1963; Pletcher et al., 1964).

(5-hydroxytryptophane) - 5HTP

5HTP is the immediate precursor of 5HT in the biosynthetic pathway of this monoamine. Injection of 5HTP will increase the amounts of 5HT in brain tissue (Undenfriend et al., 1956).

L-Dopa

Dopa is the precursor to dopamine, norepinephrine and epinephrine in the biosynthetic pathways of these biogenic amines. Injection of L-Dopa will increase the levels of these substances in brain tissue (Wiegand and

Perry, 1961; Guroff et al., 1961; Yoshida et al., 1963). Nonspecific decarboxylation by which dopamine is formed from dopa occurs throughout the brain and the regional distribution of the decarboxylases generally follows that of catecholamines (Bertles and Rosengren, 1959).

Chapter III

EXPERIMENTAL DESIGN

The purpose of this experiment was to administer drugs which would selectively raise or lower the levels of the catecholamines and/or serotonin while carefully noting the behavioral results. On the basis of the literature and previous pilot runs, dosages were used which were found not fatal to the mice. The control "city" study (Exp. I) represents an average of 20 "city" experiments. There was no appreciable difference between consecutive similar runs. An occasional hyperactive, aggressive, or sleepy mouse did not appreciably change the average number of counts per "city" experiment from one experiment to another in the controls or drug experiments. The behavior of the animals is given for each experiment as a numerical index (average number of checks per experiment per mouse). Table I lists experimental numbers, the drugs used and the number of "mouse city" runs and animals used in each experiment.

EXPERIMENT NO.	DRUGS	NO. OF "MOUSE CITIES"	NO. OF ANIMALS PER "CITY"
I	Saline	20	12
II	L-Dopa 50 mg/kg	20	12
III	Pargyline 100 mg/kg	20	12
IV	Pargyline 100 mg/kg L-Dopa 50 mg/kg	20	12
V	5-hydroxy-tryptophane 100 mg/kg	20	12
VI	Pargyline 100 mg/kg 5-hydroxy-tryptophane 100 mg/kg	20	12
VII	α-Methyl-p-tyrosine 80 mg/kg	20	12
VIII	Pargyline 100 mg/kg α-Methyl-p-tyrosine 80 mg/kg	20	12
IX	Pargyline 100 mg/kg α-Methyl-p-tyrosine 80 mg/kg 5-hydroxy-tryptophane 100 mg/kg	20	12

TABLE I

EXPERIMENT NO.	DRUG	NO. OF "MOUSE CITIES"	NO. OF ANIMALS PER "CITY"
X	α-Methyl-p-tyrosine 80 mg/kg 5-hydroxy-tryptophane 100 mg/kg	20	12
XI	Pargyline 100 mg/kg α-Methyl-p-tyrosine 80 mg/kg L-Dopa 50 mg/kg	20	12
XII	α-Methyl-p-tyrosine 80 mg/kg L-Dopa 50 mg/kg	20	12
XIII	-Parachlorophenylalanine 316 mg/kg	20	12
XIV	-Parachlorophenylalanine 316 mg/kg α-Methyl-p-tyrosine 80 mg/kg	20	12
XV	-Parachlorophenylalanine 316 mg/kg Pargyline 100 mg/kg 5-hydroxytryptophane 100 mg/kg α-Methyl-p-tyrosine 80 mg/kg	20	12

TABLE I

Evaluation of Data

The biogenic amine data was analyzed for significance using an unpaired student-t-test comparing each experiment with the control. The higher variance among animals receiving drugs is possibly due to added sources of error. The rate of absorption of the drug, and the rate of synthesis of catecholamines and serotonin may vary from animal to animal, but the results were sufficiently consistent to permit a significant statistic.

The data from "mouse city" are presented as the average behavioral trait observed per "city" per mouse (Plus or minus the standard deviation). It is difficult to apply statistical analysis to some behaviors, e.g. sexual behavior, and contactual behavior as these are rarely seen in the experimental situation. Occasionally a homosexual mouse may give the average sexual behavior a high number, but the standard deviation is also high. Those behaviors however which are shown by all animals during every experiment, e.g. exploration, provide satisfactory data for statistical analysis. Generally with such data the standard deviation ranges to about 10% of the control values (Scudder et al., 1969). With the introduction of drugs into the experiment, new behaviors occur sporadically, replacing the usual behaviors. The new behaviors are obviously significant and these as well as the orderly decreases and increases seen in the frequencies of the more usual behavioral acts are the main variables of such experiments, the data are presented here with confidence that similar trends would occur on further replication of the experiments.

The results of the biogenic amine analysis are given in Table II.

A total of 15 separate determinations were run per each experiment. Not much importance can be attached to the epinephrine levels as the measurement of this amine is at the limit of the technique. Enormous differences were encountered between treatment groups as to the levels of catecholamines and serotonin. These changes were in agreement with the specific drug effects expected on the basis of information provided by the literature and presented in the above experimental design section. Thus a monoamine oxidase (MAO) inhibitor, (Pargyline) followed by a serotonin precursor, (5-hydroxytryptophane) (5HTP), gave a large serotonin (5HT) increase. Similarly Pargyline (MAOI) followed by a NE precursor, L-Dopa, gave a large increase in dopamine and NE. A depleter of NE, (AMPT), removed selectively almost all of the NE. A serotonin depleter (CP-10) removed almost all serotonin selectively in some of the experiments. Biochemical results of drug combinations were difficult to predict as stated in the experimental design. It was in these cases of less commonly studied drug combinations that amine levels and behavior are probably not causally related was substantiated.

It is not easy to present a reader with the many correlations which such data allows. To lessen confusion and augment reading, each experiment involving either a control situation or a specific drug treatment is discussed separately in the order below and a general discussion of the entire picture stressing correlative similarities or relationships will follow (Chapter V).

Chapter IV

RESULTS

Table II represents the levels of DA, NE and 5HT found in whole mouse brain before and after drug treatment. The levels here are expressed in µg/gm wet ± SE weight. Experiment I is the saline control group.

EXPERIMENT	DA	NE	5HT
I	.875 ± .018	.465 ± .021	.650 ± .060
II	1.330 ± .168	.628 ± .010	.566 ± .016
III	1.986 ± .185	.604 ± .010	1.822 ± .165
IV	4.573 ± .210	1.023 ± .019	2.762 ± .142
V	.910 ± .019	.452 ± .016	1.462 ± .100
VI	1.967 ± .103	.797 ± .021	3.812 ± .169
VII	.010 ± .012	.010 ± .010	.699 ± .142
VIII	.657 ± .016	.317 ± .018	3.170 ± .201
IX	.744 ± .050	.303 ± .012	3.650 ± .198
X	.014 ± .010	.050 ± .014	2.080 ± .019
XI	1.050 ± .260	.744 ± .126	2.600 ± .169
XII	.700 ± .198	.349 ± .010	.663 ± .101
XIII	.832 ± .091	.469 ± .025	.260 ± .014
XIV	.009 ± .012	.028 ± .065	.260 ± .016
XV	.041 ± .086	.009 ± .014	2.110 ± .162

TABLE II

Experiment I

In an average saline control "city" experiment the animals showed the following behaviors:

TRAIT	NUMERICAL INDEX Average number of checks per experiment per mouse ± Sd.
Exploring	7.0 ± .8
Digging	0.3 ± .3
Stereotypic Behavior	0.1 ± .1
Sleeping	2.0 ± .1
Freezing	0.1 ± .2
Contactual Behavior	0.1 ± .1
Ingestion	0.5 ± .3
Sexual Behavior	0.0
Grooming Self	2.0 ± .2
Being Groomed	0.2 ± .2
Grooming Other	0.5 ± .3
Aggression	1.5 ± .4

Chart 1

Experiment I

These data represents averages from 20 "city" experiments in which the animals were injected with saline. Saline administration has, of course, a slight depressant action (Meier and Huff, 1962).

If the pre-treatment (housing and nature of aggregation) of the mice is carefully standardized the behavior of the animals in the "city" is quite constant. Occasionally a very aggressive, homosexual, or stereotypic animal is encountered.

All subsequent behavioral experiments of this study were compared with these mean control values. Generally, mice have high values for exploration and low values for other traits.

Certain other abnormal behaviors e.g. writhing, paralysis, and catatonia appear in drugged mice and are not entered on this chart (Chart 1).

Experiment II

DOSAGES AND DRUGS

L-Dopa 50 mg/kg

L-Dopa 15 minutes before experiment

AMINE	BIOGENIC AMINE LEVELS % CHANGE FROM CONTROL
DA	+ 55 $p<.01$
NE	+ 35 $p<.01$
E	+ 5 not sig.
5HT	- 3 not sig.

Chart 2

Experiment II

COMPARISON WITH CONTROL "CITY EXPERIMENTS

TRAIT	NUMERICAL INDEX	
	CONTROL	DRUGGED
Exploration decreased	7.0 ± .8	0.5 ± .3
Digging decreased	0.3 ± .3	0.0
Stereotypic Behavior decreased	0.1 ± .1	0.0
Sleeping increased	2.0 ± .1	2.5 ± .4
Freezing decreased	0.1 ± .2	0.0
Contactual Behavior increased	0.1 ± .1	0.4 ± .4
Ingestion decreased	0.5 ± .3	0.0
Sexual Behavior stayed the same	0.0	0.0
Grooming Self decreased	2.0 ± .2	1.0 ± .2
Being Groomed decreased	0.2 ± .2	0.0
Grooming Other decreased	0.5 ± .3	0.0
Aggression decreased	1.5 ± .4	0.0
Catatonia increased	0.0	10.0 ± .2

Chart 2a

Experiment II

For the first hour of the experiment the animals were in a catatonic state. They held their tails erect and rigid, resembling a "Straub" tail. The mice were seemingly not aware of each other or of their environment. The animals upon being placed in the "city" would assume a rigid position and hold it no matter how awkward the position appeared. After the first hour the mice relaxed to a resting-sleeping position, never going through the excited exploratory phase usually seen when an animal is placed in a novel environment. Nothing unusual was noticed in their physical appearance. Body temperature was normal (42 degrees C) for this strain.

Since there has been a slight 5HT decrease a large DA and NE increase in these animals, the amine changes may, presumably, be correlated with the catatonia and depression. Some authors have considered a change in sleeping time to relate to an increase 5HT line (Jouriet, 1969). The possible causal effects of the amines and serotonin on the behavioral effects seen in this experiment will be discussed later (general discussion).

Experiment III

DOSAGES AND DRUGS

Pargyline 100 mg/kg

Pargyline		24 hours before experiment Biogenic Amine levels % change from controls
DA	+ 127	P.<.01
N	+ 30	P.<.01
E	- 3	not sig.
5HT	+ 222	P.2.01

Chart 3

Experiment III

COMPARISON WITH CONTROL "CITY" EXPERIMENTS

TRAIT	NUMERICAL INDEX	
	CONTROL	DRUGGED
Exploration stayed the same	7.0 ± .8	6.5 ± .5
Digging stayed the same	0.3 ± .3	0.2 ± .2
Stereotypic Behavior stayed the same	0.1 ± .1	0.1 ± .1
Sleeping decreased	2.0 ± .1	1.5 ± .2
Freezing stayed the same	0.1 ± .2	0.1 ± .1
Contactual Behavior stayed the same	0.1 ± .1	0.1 ± .2
Ingestion stayed the same	0.5 ± .3	0.4 ± .3
Sexual Behavior stayed the same	0.0	0.0
Grooming Self decreased	2.0 ± .2	1.8 ± .5
Being Groomed stayed the same	0.2 ± .2	0.2 ± .2
Grooming Other decreased	0.5 ± .3	0.4 ± .1
Aggression decreased	1.5 ± .4	1.0 ± .6

Chart 3a

Experiment III

There was no significant behavioral difference between this "city" run and a normal control saline injected "city" run.

The increases in NE, 5HT and DA were without effect behaviorally. These changes in amines are in excess of those changes seen when L-dopa alone was administered (Exp. II) and catatonia was seen. No catatonia was observed in these pargyline treated mice. Pargyline although a more profoundly amine modulating drug than dopa, did not affect behavior as did L-dopa alone. It was expected that the animals might show an increase in activity and irritability described by Everett (1961), but they did not even though the total amine level was doubled as compared to control. It was also expected that the animals might show an increase in stereotypic behavior described by Randrup and Munkvad (1966) for rats; however, this was not the case.

Experiment IV

DOSAGE AND DRUGS

Pargyline 100 mg/kg, L-Dopa 50 mg/kg

Pargyline, one day before experiment, L-Dopa,

15 minutes before experiment.

		BIOGENIC AMINE LEVELS % CHANGE FROM CONTROLS
DA	+ 425	$p<.01$
NE	+ 120	$p<.01$
E	+ 3	not sig.
5HT	+ 325	$p<.01$

Chart 4

Experiment IV

COMPARISON WITH CONTROL "CITY" EXPERIMENTS

TRAIT	NUMERICAL INDEX	
	CONTROL	DRUGGED
Exploration decreased	7.0 ± .8	1.0 ± .3
Digging stayed the same	0.3 ± .3	0.3 ± .4
Stereotypic Behavior decreased	0.1 ± .1	0.0
Sleeping increased	2.0 ± .1	4.0 ± .4
Freezing remained the same	0.1 ± .2	0.1 ± .1
Contactual Behavior decreased	0.1 ± .1	0.1 ± .1
Ingestion decreased	0.5 ± .3	0.0
Sexual Behavior remained the same	0.0	0.0
Grooming Self decreased	2.0 ± .2	0.5 ± .2
Being Groomed decreased	0.5 ± .2	0.0
Aggression decreased	1.5 ± .4	0.0
Catatonia increased	0.0	9.0 ± .3

Chart 4a

Experiment IV

For the first hour of the "city" experiment the mice were in a catatonic state similar to the mice described in Exp. II, after which they returned to a resting-sleeping position.

This effect has been presumed to be due to increase in norepinephrine and dopamine and is a "classic" experiment often reported in the literature (Wiegand and Perry, 1961). There was an increase in 5HT and NE; greater than that seen after either drug alone, and a large increase in DA. In the "city" environment without close packing of the mice and subsequent aggregate effects, the excitement and irritability seen by others after pargyline and L-dopa administration was absent. This suggests that the environment may interact with the animals drugged condition to produce differential behavioral effects.

The main behavioral effect seen in this experiment was a catatonic-like depression. This effect may be considered to be a direct effect of the drug, L-Dopa on the CNS. There seems to be no proof that the amines need be involved *per se* in this catatonia for catatonia was not found in subsequent experiments when dopa was not employed yet amine levels were similar. This relationship will be further discussed in a following chapter (Chapter V).

Experiment V

DOSAGES AND DRUGS

5HTP 100 mg/kg

5HTP 100 mg/kg 15 minutes before experiment

		Biogenic Amine Levels
DA	+ 4	not sig.
NE	- 3	not sig.
E	- 4	not sig.
5HT	+ 125	P.<.01

Chart 5

Experiment V

COMPARISON WITH CONTROL "CITY" EXPERIMENTS

TRIAT	NUMERICAL INDEX	
	CONTROL	DRUGGED
Exploring stayed the same	7.0 ± .8	6.0 ± .5
Digging stayed the same	0.3 ± .3	0.4 ± .3
Stereotypic stayed the same	0.1 ± .1	0.1 ± .1
Sleeping stayed the same	2.0 ± .1	2.0 ± .2
Freezing stayed the same	0.1 ± .2	0.1 ± .1
Contactual stayed the same	0.1 ± .1	0.1 ± .1
Ingestion stayed the same	0.5 ± .3	0.4 ± .1
Sexual Behavior stayed the same	0.0	0.0
Grooming Self stayed the same	2.0 ± .2	1.6 ± .5
Being Groomed stayed the same	0.2 ± .2	0.1 ± .1
Grooming Others stayed the same	0.5 ± .3	0.4 ± .2
Aggression stayed the same	1.5 ± .4	1.0 ± .6

Chart 5a

Experiment V

The behavior of the animals receiving 5HTP was in no way different from the behavior of the saline treated control animals. Joyce and Mrosovsky (1964) found a significant decrease in food and an increase in water intake, and a motor retardation in rats receiving 5HTP. It was expected, therefore, that in the present experiment a change in ingestion might be seen. This was not the case, however. Nor was any motor impairment seen in this experiment as reported often in the literature (Joyce and Hurtwitz, 1964). The changes in behavior found by Joyce and co-workers will be further discussed in the following section (Exp. VI and Chapter V).

Experiment VI

Pargyline 100 mg/kg 5HTP 100 mg/kg

Pargyline - 24 hours before experiment: 5HTP 15 minutes before experiment

	Biogenic Amine Levels % change from controls	
DA	+ 126	P.<.01
NE	+ 50	P.<.01
E	0	
5HTP	+ 425	P.<.01

Chart 6

Experiment VI

COMPARISON WITH CONTROL "CITY" EXPERIMENTS

TRAIT	NUMERICAL INDEX	
	CONTROL	DRUGGED
Exploration decreased	7.0 ± .8	2.0 ± .2
Digging decreased	0.3 ± .3	0.0
Stereotypic Behavior decreased	0.1 ± .1	0.0
Sleeping decreased	2.0 ± .1	0.0
Freezing decreased	0.1 ± .2	0.0
Contactual Behavior decreased	0.1 ± .1	0.0
Ingestion decreased	0.5 ± .3	0.0
Sexual Behavior stayed the same	0.0	0.0
Grooming Self decreased	2.0 ± .2	0.0
Being Groomed decreased	0.2 ± .2	0.0
Grooming Other decreased	0.5 ± .3	0.0
Aggression decreased	1.5 ± .4	0.0
Paralysis	0.0	15.0 ± .1

Chart 6a

Experiment VI

All animals seemed paralyzed in the hind limbs and this led to an almost complete behavioral deficit. Their forelimbs and hindlimbs were abducted in a "spread eagle" fashion. The animals were tranquil for most of the experiment. During the last fifteen minutes of the experiment the animals assumed a resting position. Three (3) of the animals had slight respiratory problems characterized by hacking and irregular breathing. The animals seemed normal until the 5HTP injection.

The abduction or characteristic posture assumed by 5HTP treated mice may be due to the large serotonin increase which is the notable effect of this drug treatment although admittedly DA and NE increased also. This increase was less than that of previous experiments and cannot be held accountable for the unusual neuromuscular deficit. "Spread eagle" abduction has been reported to occur in animals after 5HTP with or without pargyline pretreatment (Everett, 1959). As described in Exp. V, motor deficit was the basis for the effect of 5HTP on avoidance behavior in rats (Joyce and Hurtwitz, 1964). Joyce and Mrosovsky (1964) reported increased drinking associated with high central levels of 5HT in mice. We saw no such behavioral change, in fact injestion decreased in our experiments. Joyce suggested many factors, from osmoreceptor effects to emotionality changes, to account for the increased water consumption. Important is her reference to Toman (1963) that the direct action of the amino acid precursor, 5HTP, may be involved. As was pointed out before, however, no neuromuscular deficit or motor impairment was seen in our experiments after

5HTP was administered alone (Exp. V).

This paralysis seen in this experiment will be discussed in a further section (Chapter V).

Experiment VII

DOSAGES AND DRUGS

MPT 80 mg/kg

MPT: TID q 4 hours for one day, and one hour before the experiment.

DA	- 99	P.<.01
NE	- 98	P.<.01
E	- 6	not sig.
5HT	+ 3	not sig.

Chart 7

Experiment VII

COMPARISON WITH CONTROL "CITY" EXPERIMENTS

TRAIT	NUMERICAL INDEX	
	CONTROL	DRUGGED
Exploration decreased	7.0 ± .8	2.0 ± .6
Digging decreased	0.3 ± .3	0.2 ± .1
Stereotypic Behavior decreased	0.1 ± .1	0.0
Sleeping increased	2.0 ± .1	10.0 ± .4
Freezing stayed the same	0.1 ± .2	0.1 ± .1
Contactual Behavior increased	0.1 ± .1	2.0 ± .4
Ingestion stayed the same	0.5 ± .3	0.4 ± .5
Sexual Behavior stayed the same	0.0	0.0
Grooming Self decreased	2.0 ± .2	1.6 ± .5
Being Groomed stayed the same	0.2 ± .2	0.2 ± .3
Grooming Other decreased	0.5 ± .3	0.3 ± .1
Aggression decreased	1.5 ± .4	0.0

Chart 7a

Experiment VII

All of the animals were depressed and most slept throughout the experiment. Piloerection was noticed during the first fifteen (15) minutes of the experiment occurring in 50% of the animals. No trembling was noticed, however. When placed in the "city" the animals were wet ventrally. Body temperature, however, was normal for this strain (42 degrees C). A viscous substance appeared around the genitalia.

These animals had suffered a considerable loss of NE and DA. They were depressed, sleeping was much increased as was contactual behavior. We thought that perhaps the low NE and DA level was causally related to the behavioral depression. A direct relationship between low NE and sleep is the only consistant one from the data presented so far. Spector et al. (1965), Moore (1967, 1968) and Hanson (1965) reported that αMPT produces overt sedation and depression of conditioned behavioral responses. Weissman and Koe (1965), however, suggested that the depressant effects of αMPT were secondary to the toxicity of the drug. Rech et al. (1966) using multiple i.p. injections of αMPT found that the drug caused impaired avoidance responding, retarded performance and spontaneous locomotor activity without producing obvious toxic effects. The content of αMPT in the brain and depletion of brain norepinephrine and dopamine both exhibited a time course similar to that of the behavioral depression. Moore (1968) found a depression in avoidance conditioning of guinea pigs following αMPT administration and related the depression to the early CA depleting

effects of MPT.

The depression and sleep seen in this experiment will be discussed in a following section with regard to the causal effects of NE and DA on the behavioral effects seen (Chapter V).

Experiment VIII

Pargyline 100 mg/kg. ⍙MPT 80 mg/kg. Pargyline 24 hours before experiment. ⍙MPT. TID q 4 hours for one day and one hour before experiment.

		Biogenic Amine Levels % change from controls
DA	- 25	P.<.01
NE	- 32	P.<.01
E	- 2	not sig.
5HT	+ 380	P.<.001

Chart 8

Experiment VIII

COMPARISON WITH CONTROL "CITY" EXPERIMENTS

TRAITS	NUMERICAL INDEX	
	CONTROL	DRUGGED
Exploration stayed the same	7.0 ± .8	6.5 ± .5
Digging stayed the same	0.3 ± .3	0.3 ± .1
Stereotypic Behavior stayed the same	0.1 ± .1	0.1 ± .1
Sleeping decreased	2.0 ± .1	0.0
Freezing stayed the same	0.1 ± .2	0.1 ± .1
Contactual Behavior increased	0.1 ± .1	0.2 ± .1
Ingestion stayed the same	0.5 ± .3	0.5 ± .2
Sexual Behavior increased	0.0	0.2 ± .1
Grooming Self decreased	2.0 ± .2	1.5 ± .6
Being Groomed decreased	0.2 ± .2	0.1 ± .1
Grooming Other decreased	0.5 ± .3	0.4 ± .1
Aggression decreased	1.5 ± .4	1.4 ± .6

Chart 8a

Experiment VIII

All animals appeared as normal control animals. Nothing unusual was noticed in their appearance. Body temperature was normal (42 degrees C) for this strain.

The Pargyline apparently counteracts the αMPT sleep producing effects observed in Exp. VII. Concurrently it prevented to a certain extent a depletion of NE and DA. Presumably the sedation, contactual behavior, and secretions seen in the previous study are due either to a very low NE or DA levels or a blockade of the direct toxic action of αMPT by pargaline. The increase in 5HT by pargeline was seen previously (Exp. III) and is without behavioral effect.

Experiment IX

DOSAGES AND DRUGS

Pargyline 100 mg/kg, ⍙MPT 80 mg/kg, 5HTP 100 mg/kg.

Pargyline 24 hours before experiment. MPT TID q 4 hours for one day and one hour before experiment.

5HPT 15 minutes before experiment.

		BIOGENIC AMINE LEVELS % CHANGE FROM CONTROL
DA	- 15	P.<.01
NE	- 35	P.<.01
E	- 2	not sig.
5HT	+ 400	P.<.001

Chart 9

Experiment IX

COMPARISON WITH CONTROL "CITY" EXPERIMENTS

TRAITS	NUMERICAL INDEX	
	CONTROL	DRUGGED
Exploration decreased	7.0 ± .8	0.0
Digging decreased	0.3 ± .3	0.0
Stereotypic Behavior decreased	0.1 ± .1	0.0
Sleeping decreased	2.0 ± .1	0.0
Freezing decreased	0.1 ± .2	0.0
Contactual Behavior decreased	0.1 ± .1	0.0
Ingestion decreased	0.5 ± .3	0.0
Sexual Behavior stayed the same	0.0	0.0
Grooming Self decreased	2.0 ± .2	0.0
Being Groomed decreased	0.5 ± .2	0.0
Grooming Other decreased	0.5 ± .3	0.0
Aggression decreased	1.5 ± .4	0.0
Paralysis increased	0.0	15.0 ± .3

Chart 9a

Experiment IX

All animals were extensively paralyzed. This resulted in a complete behavioral deficit. Both forelimbs and hindlimbs were abducted in "spread eagle" fashion. All animals seemed irritable and excited. There appeared quick, jerky movements of the head, accompanied by constant sniffing of the ground. Piloerection was noticed in two (2) animals. The animals seemed to rotate on their hindlimbs. All animals had respiratory problems characterized by hacking and irregular respiration. Body temperature averaged 1 degree below normal. The paralysis seen in this experiment was much more severe than that seen in Exp. VI.

The animals appeared normal before the injection of 5HTP, NE and DA were decreased but 5HT was much increased. The behavioral effect may be presumed due to the 5HT increase - yet this increase was less than the 5HT level reached by an injection of 5HTP and pargyline together: (Exp. VI). No amine of 5HT level is therefore a really sound explanation for this toxicity in that different drugs (discussed in Chapter V) produced similar levels of amines and 5HT without such a toxic or behavioral syndrome. Since the neuromuscular deficit was so great, a potentiation of direct 5HTP toxicity seems the most logical explanation because the 5HT level causing this pronounced paralysis is less than that causing a paralysis which was not quite so severe. On the other hand, perhaps the combination of a quite high level of serotonin with suitable changes in the other amine levels may account for the effect seen. In this experiment the pronounced

excitation occurred when both NE and DA were low. Such an effect would not be expected if low NE caused sedation as discussed in Exp. VII and VIII. This indicates that brain NE levels are not a reliable index of excitement as was postulated previously in this series of experiments unless, of course, the high 5HT counteracted the sedating effect of low NE and DA. The paralysis seen in this experiment will be discussed in a following section (Chapter V).

Experiment X

DOSAGE AND DRUGS

MPT 80 mg/kg. 5HTP 100 mg/kg

MPT: TID q 4 hours for one day and one hour before experiment.

5HTP 15 minutes before experiment.

	Biogenic Amine Levels % change from controls	
DA	- 95	P.<.01
NE	- 85	P.<.01
E	- 2	not sig.
5HT	+ 220	P.<.01

Chart 10

Experiment X

COMPARISON WITH CONTROL "CITY" EXPERIMENTS

TRAITS	NUMERICAL INDEX	
	CONTROL	DRUGGED
Exploration decreased	7.0 ± .8	4.0 ± .3
Digging stayed the same	0.3 ± .3	0.2 ± .1
Stereotypic Behavior stayed the same	0.1 ± .1	0.1 ± .1
Sleeping increased	2.0 ± .1	4.0 ± .4
Freezing stayed the same	0.1 ± .1	0.1 ± .1
Contactual Behavior increased	0.1 ± .2	1.0 ± .5
Ingestion decreased	0.5 ± .3	0.4 ± .1
Sexual Behavior stayed the same	0.0	0.0
Grooming Self decreased	2.0 ± .2	0.0
Being Groomed decreased	0.2 ± .2	0.0
Grooming Other decreased	0.5 ± .3	0.0
Aggression decreased	1.5 ± .4	0.0

Chart 10a

Experiment X

All of the animals were very tranquil and quiet during this series of experiments. They assumed a resting-sleeping position throughout most of the "city" runs. The animals were not different from control mice except for the α-MPT effect described earlier; (writhing, ventral wetting and depression). No ataxia was observed during the "city" study.

Biochemically, 5HT was increased and all amines decreased. The animals slept for most of the experiment and behaved similar to those animals given α-MPT alone (Exp. VII) although sleeping was less in this series. The sleeping behavior seen in this experiment will be discussed in Chapter V.

Experiment XI

Pargyline 100 mg/kg, α-MPT 80 mg/kg. L-Dopa 50 mg/kg.

Pargyline 25 hours before experiment.

α-MPT TID q 4 hours for one day and one hour before experiment.

L-Dopa 15 minutes before experiment

		Biogenic Amine Levels % change from control
DA	+ 20	P.<.01
NE	+ 60	P.<.01
E	+ 2	not sig.
5HT	+ 300	P.<.01

Chart 11

Experiment XI

COMPARISON WITH CONTROL "CITY" EXPERIMENTS

TRAITS	NUMERICAL INDEX	
	CONTROL	DRUGGED
Exploration decreased	7.0 ± .8	1.0 ± .4
Digging decreased	0.3 ± .3	0.2 ± .1
Stereotypic Behavior decreased	0.1 ± .1	0.0
Sleeping decreased	2.0 ± .1	0.0
Freezing decreased	0.1 ± .2	0.0
Contactual Behavior decreased	0.1 ± .1	0.0
Ingestion decreased	0.5 ± .3	0.0
Sexual Behavior decreased	0.0	0.0
Grooming Self decreased	2.0 ± .2	0.0
Being Groomed decreased	0.2 ± .2	0.0
Grooming Other decreased	0.5 ± .3	0.0
Aggression decreased	1.5 ± .4	0.0
Catatonia increased	0.0	10.0 ± .3

Chart 11a

Experiment XI

All of the animals assumed a catatonic state after the injection of L-Dopa and this accounts for the behavioral deficit. When placed in the "city" they would hold the position in which they were placed. After the first forty-five (45) minutes the animals began to walk and assume different positions which they would then hold for long periods of time. Pilo-erection was noticed during the first fifteen (15) minutes. Body temperature was normal. The animals moved slowly with no ataxia.

No amine in this experiment exceeded levels which in other experiments were correlated with toxic effects. However catatonia was observed. Therefore the catatonia is possibly not due to amine levels but some other direct action of L-Dopa on the CNS.

Experiment XII

DOSAGE AND DRUGS

αMPT 80 mg/kg. L-Dopa 50 mg/kg.

αMPT - TID q 4 hours for one day and one hour before experiment.

L-Dopa - 15 minutes before experiment.

		Biogenic Amine Levels % change from control
DA	- 20	P.<.01
NE	- 25	P.<.01
E	- 6	not sig.
5HT	+ 2	not sig.

Chart 12

Experiment XII

COMPARISON WITH CONTROL "CITY" EXPERIMENTS

TRAIT	NUMERICAL INDEX	
	CONTROL	DRUGGED
Exploration decreased	7.0 ± .8	1.0 ± .1
Digging decreased	0.3 ± .3	0.0
Stereotypic Behavior	0.1 ± .1	0.0
Sleeping increased	2.0 ± .1	8.0 ± .3
Freezing decreased	0.1 ± .2	0.0
Contactual Behavior increased	0.1 ± .1	1.0 ± .2
Ingestion stayed the same	0.5 ± .3	0.4 ± .1
Sexual Behavior stayed the same	0.0	0.0
Grooming Self decreased	2.0 ± .2	0.0
Being Groomed decreased	0.2 ± .2	0.0
Grooming Other decreased	0.5 ± .3	0.0
Aggression decreased	1.5 ± .4	0.0

Chart 12a

Experiment XII

All animals slept for most of this experiment. They were moving slowly, but were not in a catatonic state. No ataxia was observed. The animals were wet ventrally when placed in the chamber, but body temperature was normal (42 degrees C) for this strain. A viscous substance, appeared around the genitalia. No piloerection or shaking was observed.

Biochemically a NE and DA decrease was seen. Sleeping may have been due to the low norepinephrine. It is interesting to note that the direct effect of L-Dopa (described earlier) relating to catatonia was not seen. This may have been due to the low norepinephrine or due to some other effect of αMPT which blocked the L-Dopa effect. L-Dopa clearly had a pronounced catatonic effect when used with Pargyline with or without αMPT (Exp. IV and XI respectively) and when used in the same dose alone. The catatonia seen after L-Dopa administration was prevented by αMPT when no monoamine oxidase inhibitor was given seemingly irrespective of amine or 5HT levels.

Experiment XIII

DOSAGE AND DRUGS

CP10 316 mg/kg.

CP10 daily for four consecutive days.

		Biogenic Amine Levels % change from controls
DA	- 5	not sig.
NE	- 3	not sig.
E	- 1	not sig.
5HT	- 60	P.<.01

Chart 13

Experiment XIII

COMPARISON WITH CONTROL "CITY" EXPERIMENTS

TRAITS	NUMERICAL INDEX	
	CONTROL	DRUGGED
Exploration decreased	7.0 ± .8	4.0 ± .3
Digging decreased	0.3 ± .3	0.0
Stereotypic Behavior decreased	0.1 ± .1	0.0
Sleeping remained same	2.0 ± .1	2.0 ± .2
Freezing decreased	0.1 ± .2	0.0
Contactual Behavior increased	0.1 ± .1	0.2 ± .1
Ingestion increased	0.5 ± .3	4.0 ± .4
Sexual Behavior remained the same	0.0	0.0
Grooming Self decreased	2.0 ± .2	1.0 ± .1
Being Groomed decreased	0.2 ± .2	0.0
Grooming Other decreased	0.5 ± .3	0.0
Aggression remained the same	1.5 ± .4	1.5 ± .6

Chart 13a

Experiment XIII

The animals were depressed throughout the experiment. No ataxia was noticed. No piloerection or shaking occurred. Body temperature was normal (42 degrees C) for this strain.

5HT level decreased markedly, as expected. A correlation between 5HT and sleep is discussed in a following section (Chapter V).

One specific and interesting finding is the great increase in ingestive behavior. Eating has been related to brain norepinephrine stimulation; drinking to a cholinergic system (Miller, 1965). CP-10 did not increase any of the adrenergic or serotonergic transmitters but it did increase appetitive behavior. It must be presumed that the increased ingestion was not the result of a change in amine level by CP-10, but rather it is a direct drug effect.

CP-10 has been reported to facilitate sexual mounting behavior in rats and rabbits (Tagliamonte et al., 1969; Ferguson et al., 1970). These studies indicated that CP-10 may heighten homosexual mounting behavior. Later studies showed however that CP-10 would not heighten male-female sexual mounting and may alter the males ability to distinguish appropriate sexual partners (Whalen and Luttage, 1970). Our data do not substantiate facilitation of homosexual behavior in male mice.

Experiment XIV

DOSAGES AND DRUGS

CP10 316 mg/kg. ⍙MPT 80 mg/kg

CP10 daily for four consecutive days.

⍙MPT - TID q 4 hours for one day and one hour before experiment.

		Biogenic Amine Levels % Change from Controls
DA	- 99	P.<.01
NE	- 95	P.<.01
E	- 10	not sig.
5HT	- 60	P.<.01

Chart 14

Experiment XIV

COMPARISON WITH CONTROL "CITY" EXPERIMENTS

TRAITS	NUMERICAL INDEX	
	CONTROL	DRUGGED
Exploration decreased	7.0 ± .8	4.0 ± .3
Digging decreased	0.3 ± .3	0.0
Stereotypic Behavior decreased	0.1 ± .1	0.0
Sleeping increased	2.0 ± .1	9.0 ± .2
Freezing decreased	0.1 ± .2	0.0
Contactual Behavior decreased	0.1 ± .1	0.0
Ingestion increased	0.5 ± .3	3.0 ± .9
Sexual Behavior remained the same	0.0	0.0
Grooming Self decreased	2.0 ± .2	0.0
Being Groomed decreased	0.2 ± .2	0.0
Grooming Other decreased	0.5 ± .3	0.0
Aggression decreased	1.5 ± .4	0.0

Chart 14a

Experiment XIV

All of the animals were depressed throughout the experiment. There was much sleeping and ingestion. Piloerection was noticed in 50% of the animals for the first half-hour of the experiment. No ataxia was noticed. All of the animals were wet ventrally and a viscous substance much thicker than urine was noticed surrounding the genitalia. Body temperature was normal (42 degrees C) for this strain.

Many of the effects, described in earlier experiments, the increased appetite, the ventral wetting, increased sleeping and depression, were seen in this experiment. The ventral wetting and genital substance may be caused by αMPT, the high ingestion may be related to CP-10, the cause of sleeping and depression is obscure, and will be discussed in a following section (Chapter V).

Experiment XV

DOSAGES AND DRUGS

CP10 316 mg/kg, Pargyline 100 mg/kg. 5HT 100 mg/kg.

α-MPT 80 mg/kg

CP10: daily for four consecutive days

Pargyline: 24 hours before experiment

5HTP: 15 minutes before experiment

α-MPT: TID q 4 hours for one day and one hour before experiment

		Biogenic Amine Levels % Change from controls
DA	- 95	P.<.01
NE	- 99	P.<.01
E	- 4	not sig.
5HT	+ 226	P.<.001

Chart 15

Experiment XV

COMPARISON WITH CONTROL "CITY" EXPERIMENTS

TRAITS	NUMERICAL INDEX	
	CONTROL	DRUGGED
Exploration decreased	7.0 ± .8	0.0
Digging decreased	0.3 ± .3	0.0
Stereotypic Behavior decreased	0.1 ± .1	0.0
Sleeping decreased	2.0 ± .1	0.0
Freezing decreased	0.1 ± .2	0.0
Contactual Behavior decreased	0.1 ± .1	0.0
Ingestion decreased	0.5 ± .3	0.1
Sexual Behavior stayed the same	0.0	0.0
Grooming Self decreased	2.0 ± .2	0.0
Being Groomed decreased	0.2 ± .2	0.0
Grooming Other decreased	0.5 ± .3	0.0
Aggression decreased	1.5 ± .4	0.0

Chart 15a

Experiment XV

All animals were paralyzed in their hindlimbs. Both forelimbs and hindlimbs were abducted in a "spread eagle" fashion. All animals were extremely irritable and excited. There appeared quick, jerky movements of the head accompanied by constant sniffing of the ground. Piloerection was noticed in all animals. The animals seemed to rotate on their hindlimbs and had respiration problems characterized by hacking and irregular breathing. The animals were dry ventrally. Body temperature was normal (42 degrees C) for this strain.

Excitement and strong activity is present although the NE level is quite low: the abduction presumed on occasion related to serotonin is present although serotonin is lower than levels which do not cause it in other experiments. The relationship between the paralysis seen in this experiment and the pattern of amines and serotonin is discussed in the following chapter (Chapter V).

Chapter V

GENERAL DISCUSSION

In this study there are three behavioral states which need further clarification and summary. These behavioral states are catatonia, paralysis and sleeping. Because of the large amount of data the behavioral and biochemical results will be presented together on three tables.

Table III shows the drugs used, the amine concentration expressed as percent change from control, and one of the quantitated behavioral traits expressed as the average number of checks per experiment per mouse.

It can be seen from Table III that the administration of L-Dopa alone resulted biochemically in a significant increase in DA and NE. As indicated in the results section the animals were in a state of catatonia, i.e. (the animals assumed a rigid position and would not move) for an average of 10 out of 15 checks. This behavior is absent in the control animals. Administration of Pargyline alone resulted in an increase in DA, NE and 5HT in excess of that seen when L-Dopa alone was administered, yet no catatonia was seen. Perhaps the increase in 5HT seen after administration of Pargyline alone may have counteracted the catatonia resulting from the administration of L-Dopa alone. However, after the administration of Pargyline and Dopa together an increase in DA, NE, and 5HT resulted which was greater than that seen from either drug alone, yet the animals were again in a state of catatonia similar to the animals which had received L-Dopa alone. A 5HT increase had no counteracting effect. There seems to be no proof that the amines are modulating this catatonia for

nowhere in our data do we find this behavioral state without the administration of Pargyline and 5HTP resulted in an increase in DA, NE and 5HT in a manner quite similar to the catatonia producing drugs, yet no catatonia was seen. Yet catatonia was seen after the administration of Pargyline, ⍺MPT and L-Dopa, and here the amine levels are similar to those levels which did not cause catatonia. Perhaps catatonia was due to an effect of the drug, L-Dopa on the central nervous system (CNS) of the animals. However, no catatonia was seen when L-Dopa was administered with ⍺MPT. Here, however, the amines are very low. It seems, therefore, that L-Dopa has an effect of its own on the CNS: it has such an effect only when the amines are at a high optimum level.

Table IV shows that there was a significant increase in 5HT after the administration of 5HTP. The change in DA and NE, however, was not significant after this drug. DA, NE and 5HT were all significantly increased after the administration of Pargyline. The behavior of the animals receiving these two drugs alone was in no way different from the behavior of the saline control animals (Exps. III, V). However, all the animals receiving these two drugs together (Pargyline and 5HTP) exhibited a tremor of the head and forepaws as described in the results section. The animals lay in a "spread eagle" fashion for most of the experiment. Although the levels of DA, NE and 5HT are all increased with this combination, the increase in 5HT is greater than that produced by 5HTP or Pargyline alone. A tremor and paralysis was also produced as a result of the administration of Pargyline, ⍺MPT and 5HTP together. However, the DA and NE levels were reduced whereas the 5HT level was increased to 400% of control. It could

be assumed at this point that the extremely high levels of 5HT (425% of control in the first case and 400% of control in the second) may be implicated as a causal factors in the paralysis. However, subsequent experiments reveal a similar change in amines after administration of Pargyline and ⍺MPT. In this case, however, no tremor or paralysis was seen although there was an increase in 5HT to 380% of the control value, and the levels of NE and DA are very similar to those levels which were accompanied by the paralysis.

It may be that 5HTP is exerting a direct effect to cause the paralysis. It has this effect only when the level of 5HT is at a critical level.

Table V shows that after the administration of ⍺MPT, the animals suffered a significant loss of NE and DA. 5HT in this experiment was not affected. Behaviorally the animals slept for an average of 10 out of 15 checks. The control value for sleeping is two out of a total of fifteen checks.

Perhaps low NE and DA was responsible for the sleep producing effect of ⍺MPT. Branchey (1970) found that a single intraperitoneal injection of 200 mg/kg d-alpha-methyl-para-tyrosine in the rat induced a significant increase in NREM sleep without affecting REM sleep. On the other hand, Ban (1969) showed that increased brain levels of DA are associated with sedative effects whereas both DA and 5HT levels were associated with hypnotic effects. The two effects may be differentiated by a marked increased 5HT level for hypnotic effects and to a lesser extent by increases of DA and NE.

The effects of administration of Reserpine on NE and DA are well known, and a direct relationship between low NE and DA levels and the sedative effects of Reserpine is presumed by many authors (Introduction). However, because Reserpine also depletes 5HT, controversy has arisen in the past over the importance of 5HT and the catecholamines in Reserpine induced sedation. However, Reserpine induced sedation without depletion of 5HT has been reported by Gal and Millard (1967).

Table III also shows that after the administration of CP10 alone no change from control in sleeping behavior was noticed. DA and NE levels were normal, but the 5HT level was decreased after administration of this drug.

After administration of CP10 and αMPT together the animals showed an increase in sleeping behavior over the control animals. The drugged animals slept for an average of nine out of a total of fifteen checks. DA, NE and 5HT were all decreased. So far then the increase in sleeping behavior occurs whenever there is an accompanying low level of both DA and NE, regardless of the levels of 5HT. The level of 5HT seems to be unimportant so far, i.e. after administration of αMPT alone it was normal and after CP10 and αMPT together it was decreased, yet in both of these experiments an increase in sleeping behavior was seen. However, after administration of Pargyline and αMPT together a decrease in sleeping was seen although DA and NE levels were again decreased and the 5HT level was increased. The administration of Pargyline, CP10 and αMPT and 5HT together produced similar changes in amines and sleeping behavior. It would seem then that the high 5HT level counteracted the sedating effect

of low DA and NE. Hery et al. (1970) found an increased 5HT synthesis induced by paradoxical sleep deprivation. This seemed to be the result of increased uptake of tryptophan as well as increased conversion to 5HT. His results supported the hypothesis that serotonergic neurons are involved in paradoxical sleep processes. Delorme et al. (1966) showed that chronic serotonin depletion by PCPA (CP10) is accompanied by a transitory insomnia, the emergence of lateral geniculate spikes into wakefulness, and global behavioral changes including hypersexuality, irritability, and increased aggressiveness.

Our hypothesis, that the high serotonin counteracted the sedative effect of low NE and DA was tenuous because the administration of αMPT and 5HTP produced a change in amines almost exactly the same as that produced by Pargyline and αMPT together. That is, a decrease in NE and DA and an increase in 5HT. Sleeping was <u>increased</u> in this case and the high 5HT level had no counteracting effect.

Sleeping may be due to a direct drug effect, i.e. αMPT produces sedation except when administered alone with Pargyline. Such a drug interaction is the only simple hypothesis this data permits. Low levels of DA and NE, then, do not seem to be a reliable index of sedation and sleep as postulated earlier.

Miller (1965) in a preliminary report suggested that the balance of free 5HT/NE was the deciding factor in behavioral depression induced by the benzoquinolizines. He suggested that the preponderance of free 5HT/NE resulted in sedation.

EXP. NO.	DRUG	AMINE CONCENTRATION % CHANGE FROM CONTROL			BEHAVIOR AVERAGE NO. OF CHECKS PER EXP. PER MOUSE ± S.D.
		DA	NE	5HT	CATATONIA
I	SALINE CONTROL	0	0	0	0
II	L-DOPA	+55+	+35+	-3*	10 ± .2
III	PARGYLINE	+127+	+30+	+222++	0
IV	PARGYLINE L-DOPA	+425++	+120++	+325++	9 ± .3
VI	PARGYLINE 5 HTP	+126+	+50+	+425++	0
XI	PARGYLINE α MPT L-DOPA	+20+	+60+	+300++	10 ± .3
XII	α MPT L-DOPA	-20+	-25+	+2*	0

++ = P. < .001

+ = P. .01

EXP. NO.	DRUG	AMINE CONCENTRATION % CHANGE FROM CONTROL			BEHAVIOR AVERAGE NO. OF CHECKS PER EXP. PER MOUSE ± S.D.
		DA	NE	5HT	PARALYSIS
I	SALINE CONTROL	0	0	0	0
V	5HTP	+4*	−3*	+125	0
III	PARGYLINE	+127++	+30+	+222++	0
VI	PARGYLINE 5HTP	+126++	+50+	+425++	15
IX	PARGYLINE α MPT 5HTP	−15+	−35+	+400++	15
VIII	PARGYLINE α MPT	−25+	−32+	+380++	0

++ = P. < .001

+ = P. < .01

EXP. NO.	DRUG	AMINE CONCENTRATION % CHANGE FROM CONTROL			BEHAVIOR AVERAGE NO. OF CHECKS PER EXP. PER MOUSE ± S.D.
		DA	NE	5HT	SLEEP
I	SALINE CONTROL	0	0	0	2 ± .1
VII	α MPT	-99^{++}	-98^{++}	$+3^{*}$	10 ± .4
XIII	CP-10	-5^{*}	$+1^{*}$	-60^{++}	2 ± .2
XIV	CP-10 α MPT	-99^{++}	-95^{++}	-60^{++}	9 ± .2
VIII	PARGYLINE α MPT	-25^{+}	-32^{+}	$+380^{++}$	0
XV	PARGYLINE CP-10 α MPT 5 HTP	-95^{++}	-99^{++}	$+226^{++}$	0
X	α MPT 5 HTP	-95^{++}	-85^{++}	$+220^{++}$	4 ± .3
XII	α MPT L-DOPA	-20^{+}	-25^{+}	$+2^{+}$	8 ± .3

++=P. < .001

+=P. < .01

Chapter VI

CONCLUSION

It is known that microinjection of different drugs in identical brain areas will elicit different discrete behavioral acts (Miller, 1965). It has also been shown that the drug systematically injected may have a behavioral effect opposite to that of a selective placement by microinjection in the brain (Gal and Millard, 1967). Our present studies have several disadvantages: 1) The drugs are injected systemically and the observed changes in amines may differ from fluctuations in amines brought about by local injection. 2) The behavioral parameters which were studies are often obliterated by the toxic effects of individual drugs. 3) Amine changes in brain parts were not determined. Indeed, the amine levels are determined for whole brain and presumably the biogenic amine levels have changed everywhere at varying rates of synthesis or depletion, both bound and unbound. Therefore it is still possible, even if clear cut behavioral modulation is not seen in these experiments that chemical coding of behavior exists. Certainly hormonal effects on behavior are well established (Miller, 1965) and since quantitation of mental stress by an analysis of urinary catecholamine levels is possible (Von Euler, 1964), there must be a close correlation if not a causal relationship.

Behaviorally, in some parameters, widely different chemical agents may be similar in effects. Thus atropine and scopolamine, MAOI, phenothiazines, codeine and antihistaminics, may reverse aggressive behavior

at low, non-toxic doses (Da Vanzo et al., 1968). It is well known that different central acting drugs; bemegride, morphine, alcohol and many others show selective action on different centers, circuits, systems or cell populations in the brain. There may or may not be a transmitter basis for the behavioral effects of these drugs. The effects may be due to a change in irritability of certain neurons selectively.

On the basis of the lack of consistant correlation between amines of whole brain and behavior and the great fluctuations of amine levels measured in these experiments, it is unlikely that changes in the levels of the biogenic amines alone could give much insight on complex mechanisms involved in behavior. Whereas the effects of these amines at particular sites in the brain probably are of crucial importance in the regulation of behavior, any formulation of the physiology of behavior would have to include other biochemical, physiological and psychological factors.

Summary

The (whole mouse brain) levels of norepinephrine, epinephrine, serotonin, dopa and dopamine were varied more or less selectively by means of suitable injection schedules and combinations of parachlorophenylalanine L-Dopa, 5-hydroxytryptophane, pargyline and a-methyl-p-tyrosine. The amines and serotonin were extracted and determined spectrophotoflouro-metrically according to the technique of Weigand and Perry (Biochem. Pharm. 7: 181-6, 1961). The extraction was made at the mid point in time during which the behavior of similarly treated animals was quantitated.

The behavior (of the mice) was quantitated using a "Mouse City" set-up made of plexiglass. It is composed of six small home cages

(5" x 7" x 4") each connecting with a central communal chamber (18" x 11" x 6") by means of funnel-like, cylindrical runways. In the city experiment, two aggregated males, after suitable drug pre-treatment, were placed in each of the small cages of the "city". The animals were subsequently allowed to freely interact with one another and at five minute intervals were checked for the following behavioral parameters: exploration, digging, stereotypic behavior, food-carrying, sexual behavior, freezing, sleeping, contactual behavior and aggression.

No completely satisfactory correlation was found between any single amine level or ratio of amine levels and behavior. Three predominant behaviors emerged from these studies; catatonia, sleep and paralysis.

The effect of the drugs on other behaviors is discussed in reference to these three behaviors. The effects on behavior seen in these experiments by and large are presumed to be due either to direct drug effects or to effects of drugs on other neurohumors or systems not measured in this study. Catecholamines and Serotonin did not in general prove to be reliable indexes of any behavior nor did levels of the amines or serotonin give predictable behavioral responses.

The work presented in this thesis was helpful in clearing the way for a better understanding of which behaviors and what aspects of brain function are regulated by the neurohumors, and although the results are largely negative they were invaluable for a constructive interpretation in later research for a Doctorate Dissertation.

List of Illustrations

Figures

Tables

Charts

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Acceptance Sheet

The dissertation submitted by Daniel L. Richardson has been read and approved by the members of the examining committee.

The final copies of the dissertation have been examined by the chairman of the examining committee and the signature which appears below verifies the fact that all necessary changes have been incorporated and that the dissertation is now given final approval with reference to content, form and mechanical accuracy.

The dissertation is accepted in partial fulfillment of the requirements for the Degree of Master of Science.

October 18, 1971
Date

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